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EFFECTS OF METHIONINE SULFOXIMINE ON CEREBRAL ATPases

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Abstract—Hyperammonemia inducing drug, L-methionine-DL-sulfoximine (MSI), was administered to rats and the changes in the activities of Na^+, K^+-ATPase and Mg^{2+}-ATPase were followed in three different brain regions. The activity of Mg^{2+}-ATPase decreased over the control, while that of Na^+, K^+-ATPase increased in all regions. The per cent increase was more in brain stem (BS) than in cerebral cortex (CC) and cerebellum (CE). *In vitro* addition of MSI and ammonium chloride also resulted in similar changes. These results indicate that MSI induced toxicity is not only due to inhibition of glutamine synthetase but also by stimulation of Na^+, K^+-ATPase leading to the depletion of cerebral ATP levels.

INTRODUCTION

The convulsant action of methionine sulfoximine (MSI) was shown to be due to the inhibition of glutamine synthetase leading to a hyperammonemic state [1]. The increased levels of ammonia and glutamate in cerebral tissue following the administration of MSI [2] and the production of Alzheimer type II astrocytosis [3] (a constant feature of hyperammonemic states during hepatic-failure) favoured this theory. However, Warren and Schenker [4] reported an increase in the LD_{50} for exogeneously administered ammonium salts in the animals treated with MSI. These results, in conjunction with those of Folbergrova *et al.*, and others [5, 6] indicated the existence of additional mechanisms for the MSI toxicity. Based on our earlier observations, that ammonia salts stimulate the activity of Na^+, K^+-ATPase in cerebral tissues [7, 8], we have presently studied the activity of the enzyme and Mg^{2+}-ATPase in brain during MSI toxicity.

MATERIALS AND METHODS

Animals. Adult albino rats from an inbred colony of Wistar strain were chosen as experimental animals. The animals were of either sex and of same age, having a body wt of 150–200 g. Both control and experimental animals had free access to food (balanced pellet diet from Hindustan Lever Limited) and water.

Drug treatment. L-Methionine-DL-sulfoximine was administered intraperitonially using saline as a carrier. In order to bring the effects in a short span of time, a high dose schedule (300 mg/kg body wt) was followed in this study and the control animals received an equal volume of saline.

Isolation of brain regions, preparation of homogenates and methods of enzyme assay were described earlier [7, 8].

Vanadium free ATP, L-methionine-DL-sulfoximine and ouabain were purchased from Sigma Chemical Company (St. Louis, MO). Other reagents were either AnalaR or GR grade from BDH or SM (India).

RESULTS

Behavioural changes

The behavioural changes observed in the present study were more or less similar to those reported by Gutierrez and Norenberg [3] except that the time period was short due to high dosage of the drug administered. One hour after the administration of the drug, the animals showed less physical activity and this parameter decreased progressively. At 2 hr the animals exhibited a change in their normal gait to wobbly and splayed leggedness. At the onset of 3 hr the animals lost their sense of equilibrium and could not recover it when altered manually. Four hours after the administration of the drug the animals exhibited uncontrolled rolling movements along their body axis and entered into a state of tonic and clonic convulsions. The incidence of mortality was high during and after this time period.

Mg^{2+}-*ATPase* (Table 1)

One hour after the administration of MSI, the activity of Mg^{2+}-ATPase decreased significantly in CC and CE. This change in the activity of the enzyme persisted even at the end of 2 hr. The per cent change in the activity was more when expressed per g wet wt of tissue. At the end of 3.5 hr the activity returned to normal levels in CC and remained elevated in CE. However, this change could be observed only when the activity was expressed per g wet wt of tissue but was less than the control when expressed per mg protein. Throughout the time period the changes in the activity of this enzyme were less or not significant in the BS.

Na^+, K^+-*ATPase* (Table 2)

The activity of Na^+, K^+-ATPase did not show any statistically significant change in CC during the time periods studied after the administration of MSI when the activity was expressed per g wt of tissue. However, when the activity was expressed per mg

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Table 1. Distribution and levels of Mg^{2+}-ATPase in three different regions of brains of normal and experimental rats

		Cerebral cortex	Per cent over control	Cerebellum	Per cent over control	Brain stem	Per cent over control
Control	*	624 ± 83(6)		520 ± 45(7)		342 ± 91(7)	
	†	5.1 + 0.9(5)		4.6 + 0.4(7)		3.4 ± 0.8(7)	
1 hr after MSI administration	*	381 ± 39(5) P < 0.001	−39	380 ± 64(6) P < 0.001	−27	280 ± 29(6) NS	−18
	†	3.7 + 0.5(5) P < 0.001	−27	3.9 ± 0.5(6) P < 0.02	−14	2.9 ± 0.6(6) NS	−16
2 hr after MSI administration	*	388 ± 34(5) P < 0.001	−38	361 ± 54(6) P < 0.001	−30	324 ± 74(6) NS	−5
	†	4.0 ± 0.4(5) P < 0.05	−22	3.9 ± 9(6) P < 0.1	−15	3.4 ± 0.9(6)	−2
3.5 hr after MSI administration	*	657 ± 108(6) NS	+5	670 ± 99(6) P < 0.005	+29	353 ± 62(5) NS	+3
	†	3.4 ± 0.7(7) P < 0.01	−33	3.0 ± 1.0(6) P < 0.005	−17	1.9 ± 0.5(6) P < 0.01	−45

* μmoles of P_i liberated/g wet wt tissue/hr.
† μmoles of P_i liberated/mg protein/hr.
Each value is mean ± S.D.
Figures in parentheses indicate number of animals.
NS: Statistically not significant.

protein, it increased up to 2 hr after MSI administration, and at the end of 3.5 hr the activity decreased considerably. In CE the activity increased considerably over the control values up to 2 hr. At 3.5 hr the activity either restored to control value or decreased depending on the mode of expression. However, in BS, the activity showed a significant increase over control at all the time periods irrespective of mode of expression. The per cent increase in BS was higher than in the other regions at all time intervals.

In vitro *effects*

Effect of MSI on Mg^{2+}*-ATPase.* The direct effect of MSI on the activity of Mg^{2+}-ATPase was presented in Table 3. Even up to 10 μmoles, MSI did not influence the activity of this enzyme.

Effect of MSI on Na^+, K^+*-ATPase.* Addition of MSI had a differential effect on the activity of Na^+, K^+-ATPase in the three different brain regions. The enzyme in CE and BS was stimulated even at low concentrations of MSI while that in CC was stimulated only at higher drug concentrations. In BS, the per cent increase in the activity increased with increasing drug concentrations (Table 3).

Effect of ammonium ion on Mg^{2+}*-ATPase.* Unlike MSI, ammonium ion had a differential effect on the activity of Mg^{2+}-ATPase depending on the concentration and region studied. In CC only high concentrations of ammonium ion had a stimulatory effect of Mg^{2+}-ATPase activity. In CE the enzyme activity increased at both the concentrations only when the activity was expressed per mg protein. In BS the increase in the activity was observed both at 10 and 20 μmole concentrations (Table 4).

In vitro *effect of ammonium ion on* Na^+, K^+*-ATPase.* Low concentrations of ammonium ion

Table 2. Distribution and levels of Na^+, K^+-ATPase in three different regions of brains of normal and experimental rats

		Cerebral cortex	Per cent over control	Cerebellum	Per cent over control	Brain stem	Per cent over control
Control	*	155 ± 32(7)		111 ± 25(9)		70 ± 10(5)	
	†	1.3 ± 0.3(7)		1.0 ± 0.2(9)		0.6 ± 0.1(6)	
1 hr after MSI administration	*	187 ± 47(6) P < 0.01	+20	164 ± 29(6) P < 0.005	+48	176 ± 18(6) P < 0.001	+151
	†	1.9 ± 0.6(6) P < 0.01	+46	1.7 ± 0.5(6) P < 0.05	±90	1.8 + 0.3(6) P < 0.001	+200
2 hr after MSI administration	*	173 ± 22(5) NS	+12	243 ± 47(5) P < 0.001	+119	225 ± 49(4) P < 0.001	+221
	†	1.8 ± 0.2(5) P < 0.01	+38	2.4 ± 0.4(6) P < 0.001	+142	1.9 ± 0.5(6) P < 0.005	+215
3.5 hr after MSI administration	*	154 ± 52(7) NS	+0.6	127 ± 31(7) NS	+14	219 ± 84(7) P < 0.005	+200
	†	0.8 ± 0.3(7) P < 0.01	−39	0.78 ± 0.2(7) P < 0.1	−22	1.3 ± 0.4(7) P < 0.005	+116

* Footnotes as in Table 1.

Table 3. *In vitro* effects of MSI on Mg^{2+}-ATPase and Na^+, K^+-ATPase (per cent change over controls)

Region		Mg^{2+}-ATPase MSI concentration (μmoles)			Na^+, K^+-ATPase MSI concentration (μmoles)		
		1	5	10	1	5	10
Cerebral cortex	*	−11	0	+0.3	−10	−14	+28
		$P < 0.1$	NS	NS	NS	$P < 0.1$	$P < 0.05$
	†	−3	+5	+11	−6	+4	+32
		NS	NS	$P < 0.1$	NS	NS	$P < 0.05$
Cerebellum	*	+8	+15	−9	+33	+24	+22
		NS	$P < 0.01$	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$
	†	+8	+15	+9	+34	+22	+24
		NS	$P < 0.01$	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$
Brain stem	*	−7	−3	−5	+117	+193	+241
		NS	NS	NS	$P < 0.01$	$P < 0.001$	$P < 0.001$
	†	−3	−8	−11	+130	+209	+260
		NS	NS	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$

* When the activity expressed per g wet wt of tissue.
† When the activity expressed per mg of protein.
Each value indicates the per cent change over the control value: + increase; − decrease.

(10 μmoles) had no effect on Na^+, K^+-ATPase activity in CC, while at high concentrations there was a significant increase. In BS an opposite trend was observed and in CE addition of ammonium ion had no effect on Na^+, K^+-ATPase activity (Table 4).

DISCUSSION

In previous reports we demonstrated an increase in the activity of cerebral Na^+, K^+-ATPase in acute and chronic ammonia toxicity [7, 8]. In addition, we have also shown that when homogenates were enriched with ammonium ion, only the enzyme from BS was activated. This and the report of Skou and Hilberg [9], prompted us to propose that NH_4^+ stimulates cerebral Na^+, K^+-ATPase which might explain some of the neurological changes in ammonogenic coma. In the present communication, we report the same phenomenon could be induced by hyperammonemia inducing drugs such as MSI.

The enzyme, Mg^{2+}-ATPase, was routinely assayed for ouabain resistant nonspecific ATP hydrolysis in the determination of Na^+, K^+-ATPase activity. Hence, this activity might represent the activity of one or several ATP hydrolyzing enzymes requiring Mg^{2+}. The observed fall in this activity in MSI toxicity could be a compensatory phenomenon, since the major ATP consuming reaction of the brain (Na^+, K^+-ATPase) was increased considerably under these conditions. The latter enzyme, which is involved in the maintenance of ionic gradients, occupies a more pivotal position in neuronal function than the other ATP requiring reactions [10], hence more ATP could be made available to this enzyme by inhibiting the other ATP hydrolysing reactions. The exact mechanism(s) involved in bringing this effect was not

Table 4. *In vitro* effects of ammonium ion on Mg^{2+}-ATPase and Na^+, K^+-ATPase (per cent changes over control)

Region		Mg^{2+}-ATPase Ammonium ion concentration (μmoles)		Na^+, K^+-ATPase Ammonium ion concentration (μmoles)	
		10	20	10	20
Cerebral cortex	*	−16	−43	+7	+145
		NS	$P < 0.005$	NS	$P < 0.001$
	†	−20	−47	+1	+158
		$P < 0.1$	$P < 0.005$	NS	$P < 0.001$
Cerebellum	*	−21	+21	−9	−5
		$P < 0.05$	$P < 0.05$	NS	NS
	†	−21	0	−17	−2
		$P < 0.1$		NS	NS
Brain stem	*	−22	+29	+57	−22
		$P < 0.01$	$P < 0.02$	$P < 0.001$	$P < 0.05$
	†	−23	+25	+102	−5
		$P < 0.0005$	$P < 0.05$	$P < 0.01$	NS

* Footnotes as in Table 3.

clear, but the *in vitro* studies indicated that MSI mediates this change not directly but through ammonia, the cerebral levels of which were shown to be increased by the MSI.

The lack of any significant change in the activity of Na^+, K^+-ATPase in CC and a significant increase in CE and BS (when the activity was expressed per g wet weight) suggested that the drug, MSI, had differential effect on different brain regions. The *in vitro* studies also indicated that the drug could differentially stimulate the activity of Na^+, K^+-ATPase in different regions in a dose dependent manner. As in the case of Mg^{2+}-ATPase the changes in Na^+, K^+-ATPase activity might be due to the direct action of MSI or through ammonium ion. Even in *in vitro* conditions, MSI by inhibiting glutamine synthetase might lead to an accumulation of ammonia, which is generated during incubation in the homogenates [11]. To check this, we have studied the effect of ammonium ion on the enzyme activity in homogenates dialyzed overnight (against 0.32 M sucrose). It could be observed from the results that ammonium ion also exerted a differential effect on the enzyme activity in different brain regions in a dose dependent manner. Such a result would then indicate a differential nature of the enzyme protein in these regions.

The topographical differences in the drug effect, though not uncommon, might render some regions more vulnerable than the other. In the present study, it was apparent that CC was less susceptible than BS while CE occupies an intermediate position. The vulnerability of brain stem to ammonia toxicity was reported earlier and a fall in ATP levels in this region was also documented [12].

The above studies supported our earlier view, that ammonium ion could exert its toxic effect through stimulation of cerebral Na^+, K^+-ATPase. Though the exact mechanism of this stimulation was not worked out, the work of Skou [9] indicated that NH_4^+ could bring this effect in the presence of Na^+ and Mg^{2+}. In other words, NH_4^+ replaced K^+ in this reaction probably because of similarities in the sizes of these hydrated ions.

Thus our studies indicate that MSI exerts its action not only by inhibiting glutamine synthetase but also by stimulating Na^+, K^+-ATPase either acting directly on the enzyme or by elevating cerebral ammonia levels.

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